Characterization of a potent non-cytotoxic shRNA directed to the HIV-1 co-receptor CCR5.

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Public Summary:

Scientific Abstract:
BACKGROUND: The use of shRNAs to downregulate the expression of specific genes is now relatively routine in experimentation but still hypothetical for clinical application. A potential therapeutic approach for HIV-1 disease is shRNA mediated downregulation of the HIV-1 co-receptor, CCR5. It is increasingly recognized that siRNAs and shRNAs can have unintended consequences such as cytotoxicities in cells, particularly when used for long term therapeutic purposes. For the clinical use of shRNAs, it is crucial to identify a shRNA that can potently inhibit CCR5 expression without inducing unintended cytotoxicities. RESULTS: Previous shRNAs to CCR5 identified using conventional commercial algorithms showed cytotoxicity when expressed using the highly active U6 pol III promoter in primary human peripheral blood derived mononuclear cells. Expression using the lower activity H1 promoter significantly reduced toxicity, but all shRNAs also reduced RNAi activity. In an effort to identify shRNAs that were both potent and non-cytotoxic, we created a shRNA library representing all potential CCR5 20 to 22-nucleotide shRNA sequences expressed using an H1 promoter and screened this library for downregulation of CCR5. We identified one potent CCR5 shRNA that was also non-cytotoxic when expressed at a low level with the H1 promoter. We characterized this shRNA in regards to its function and structure. This shRNA was unique that the use of commercial and published algorithms to predict effective siRNA sequences did not result in identification of the same shRNA. We found that this shRNA could induce sequence specific reduction of CCR5 at post transcriptional level, consistent with the RNA interference mechanism. Importantly, this shRNA showed no obvious cytotoxicity and was effective at downregulating CCR5 in primary human peripheral blood derived mononuclear cells. CONCLUSION: We report on the characterization of a rare shRNA with atypical structural features having potent RNAi activity specific to CCR5. These results have implications for the application of RNAi technology for therapeutic purposes.